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Step I - First Strand Synthesis

m<sup>7</sup>G ——— (A)<sub>n</sub> Messenger RNA

↓ Annealing of oligo d(T) Primer

m<sup>7</sup>G ——— (A)<sub>n</sub>  
                                  -(dT)

↓ Reverse Transcriptase

m<sup>7</sup>G ——— (A)<sub>n</sub>  
                                  -(dT)

Step II - Second Strand Synthesis

DNA Polymerase I  
Ribonuclease H  
E. coli DNA Ligase

Alkaline Hydrolysis of RNA  
Terminal Transferase  
dTTP

——— (dA)<sub>n</sub> TTTTTT ——— (dT)<sub>n</sub>  
——— (dT)

↓ Oligo d(A)<sub>n</sub>  
T7 DNA Polymerase

AAAAAA ———  
TTTTTT ——— (dT)<sub>n</sub>

T4 DNA Polymerase

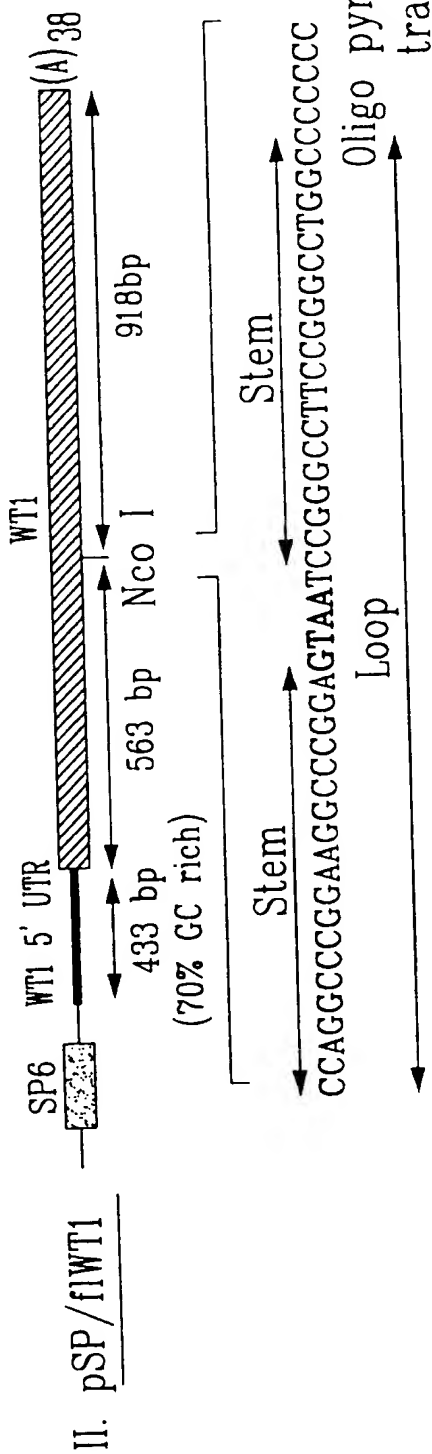
T4 Ligase  
(Adaptors)

——— (dA)  
——— (dT)

Step III - Polishing and Cloning

Clone into appropriately  
prepared vector

FIG. 1 PRIOR ART



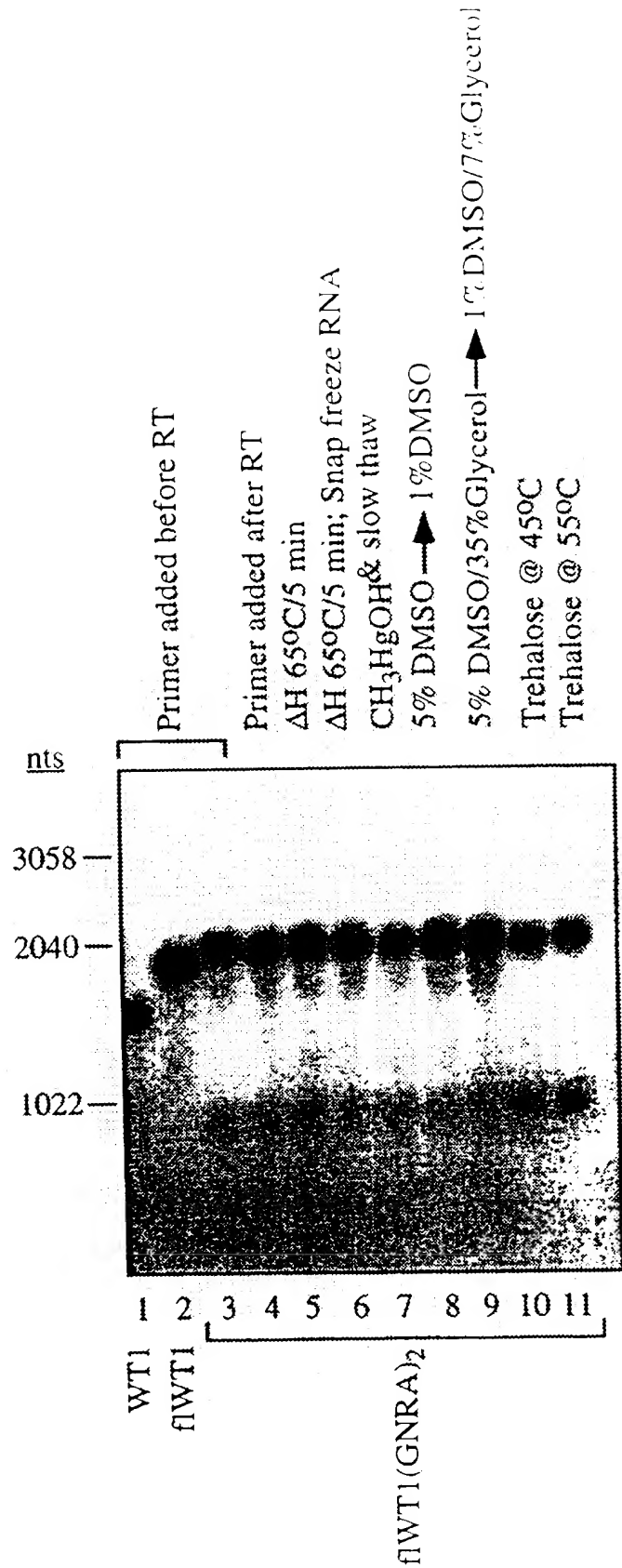
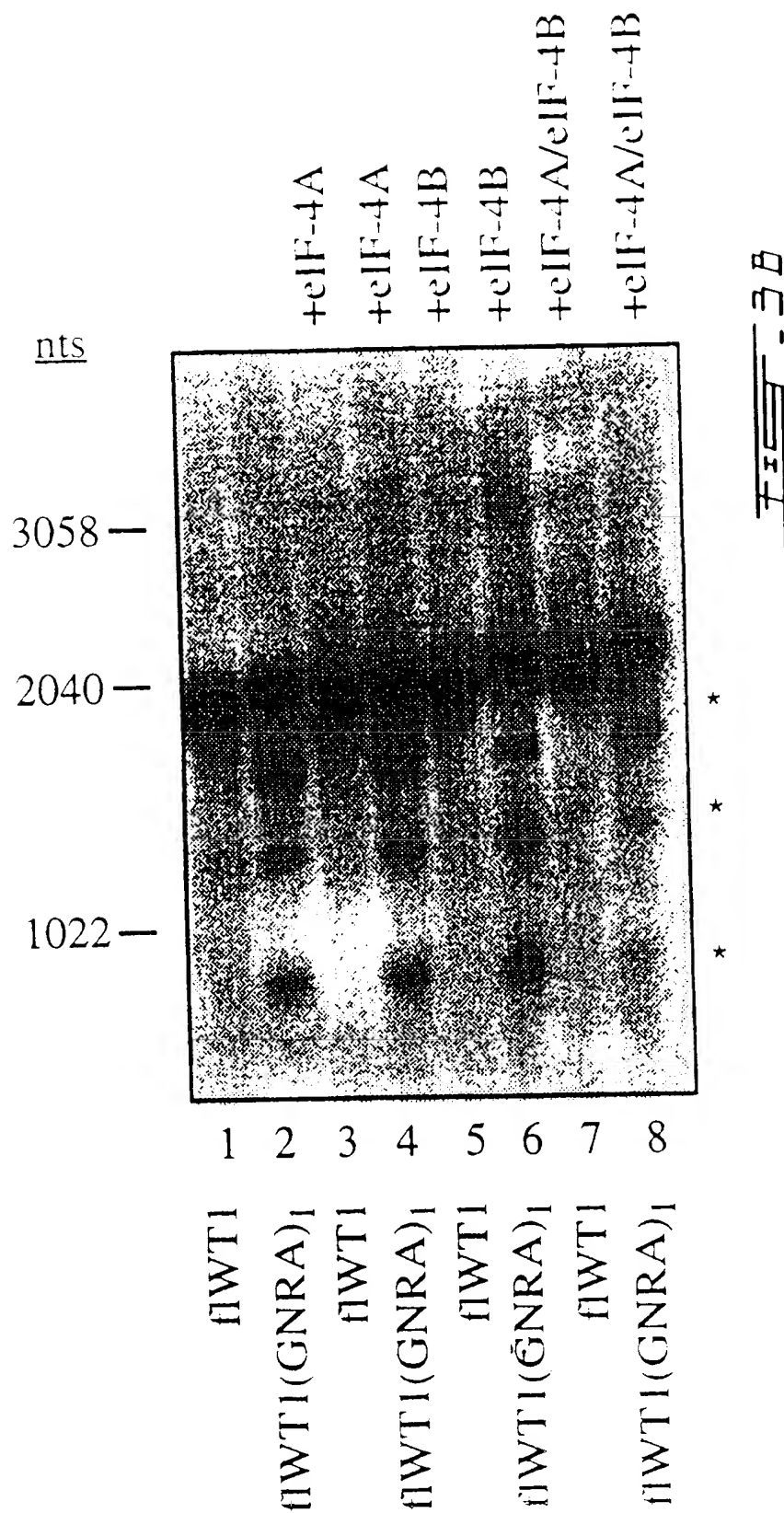


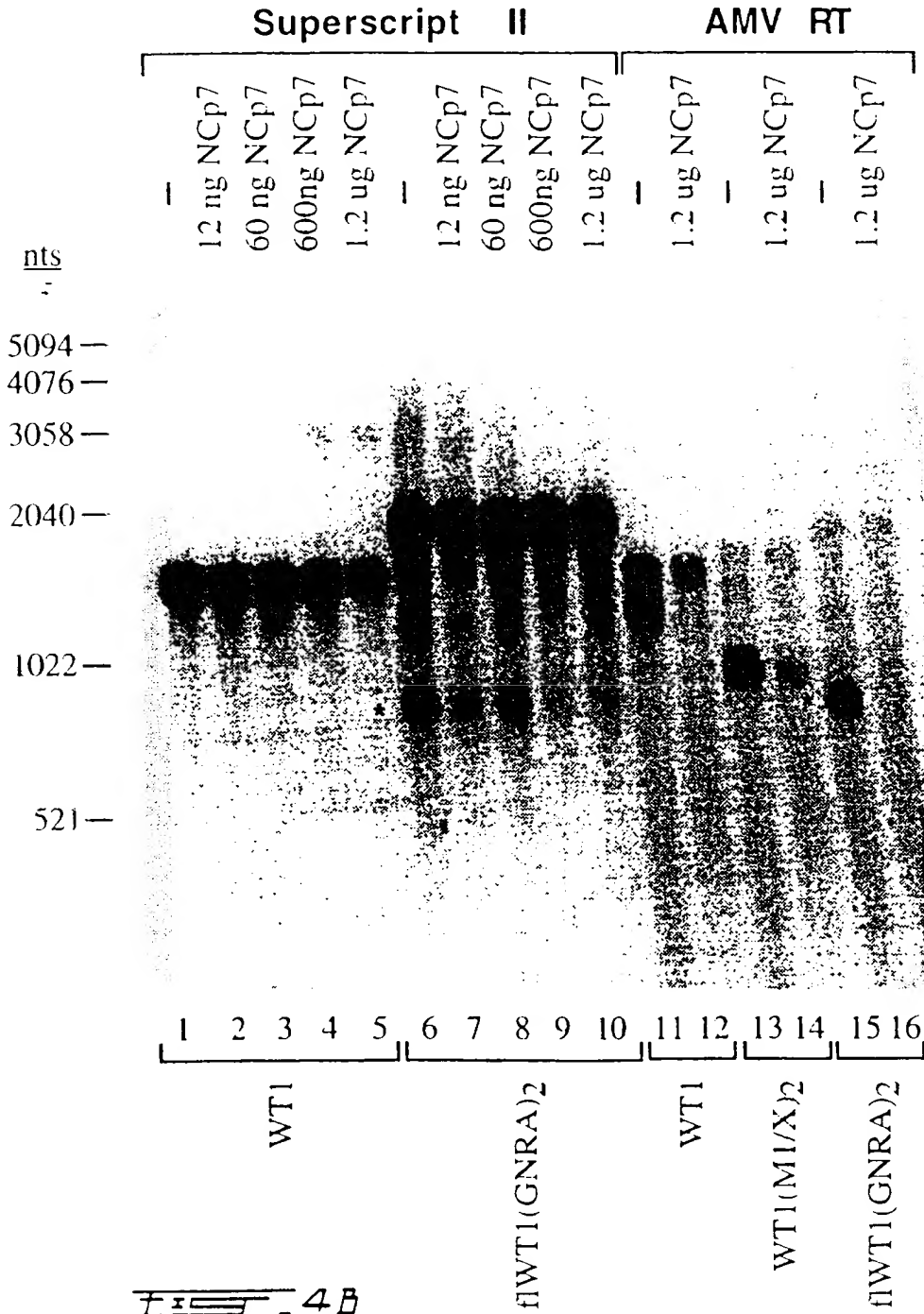
FIG. 3A



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NH<sub>2</sub>-MQRGNFRNQRKNVKCFNCGKEGHTARNCRAPRKKGCWKCCKEGHQMKDCTERQANFLGKIWPSYKGRPGNFL-COOH

Fig. 4A



*In vitro* transcribed WT1 mRNA

————— (A)<sub>n</sub>

↓ Superscript II  
Oligo d(T)<sub>n</sub>

===== (A)<sub>n</sub>  
===== (T)<sub>n</sub>

↓ Alkaline hydrolysis of RNA  
Terminal transferase  
dTTP

TTTTT ————— (T)<sub>n</sub>

↓ T7 DNA polymerase  
Oligo d(A)<sub>n</sub>  
α-<sup>32</sup>P-dATP

AAAAA — — — — —  
TTTTT ————— (T)<sub>n</sub>



**Alkaline agarose gel Analysis**

Fraser - 5A

